

WO 97/11094) by polymerase chain reaction (PCR). The PCR reactions were performed according to standard protocols with the following primers:

5'PKAc:

TTggACACAAgCTTTggACACCCTCAggATATgggCAACgCCgCCgCCgCCA

Ag (SEQ ID NO:19),

3'PKAc:

gTCATCTTCTCgAgTCTTTCAggCgCgCCCAAACCTCAgTAAACTCCTTgCCACA

C(SEQ ID NO:20)

5'GFP:

TTggACACAAgCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTT

C(SEQ ID NO:21)

3'GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT

(SEQ ID NO:22).--

Please replace the paragraph beginning on page 44, line 32 with the following amended paragraph:

--EXAMPLE 2 Probe for detection of PKC activity

Construction of PKC-GFP fusion:

The probe was constructed by ligating two restriction enzyme treated polymerase chain reaction (PCR) amplification products of the cDNA for murine PKC α (GenBank Accession number: M25811) and F64L-S65T-GFP (sequence disclosed in WO 97/11094) respectively. Taq® polymerase and the following oligonucleotide primers were used for PCR;

5'mPKCα:

TTggACACAAGCTTTggACACCCTCAggATATggCTgACgTTTACCCggCCA

ACg (SEQID NO:23)

3'mPKCα:

gTCATCTTCTCgAgTCTTTCAGgCgCgCCCTACTgCACTTTgCAAgATTgggT

gC (SEQ ID NO:24),

5'F64L-S65T-GFP:

TTggACACAAGCTTTggACACggCgCgCCATgAgTAAAggAgAAgAACTTTT

C (SEQ ID NO:25),

3'F64L-S65T-GFP:

gTCATCTTCTCgAgTCTTACTCCTgAggTTTgTATAgTTCATCCATgCCATgT

(SEQ ID NO:26).--

Please replace the paragraph beginning on page 47, line 13 with the following amended paragraph:

--The human Erk1 gene (GenBank Accession number: X60188) was amplified

using PCR according to standard protocols with primers

Erk1-top

5'-TAGAATTCAACCATGGCGGCGGCGGCGGCG (SEQ ID NO:27)-3'

and Erk1-bottom/+stop

5'-TAGGATCCCTAGGGGGCCTCCAGCACTCC (SEQ ID NO:28)-3'.

B3
cont'd

The PCR product was digested with restriction enzymes EcoR1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with EcoR1 and BamH1. This produces an EGFP-Erk1 fusion (SEQ ID NOs: 5 and 6) under the control of a CMV promoter.--

Please replace the paragraph beginning on page 48, line 14 with the following amended paragraph:

--Smad 2, a signal transducer, is a component of a signalling pathway that is induced by some members of the TGFbeta family of cytokines.

a) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:29)-3'

and Smad2-bottom/+stop

5'-GTGGTACCTTATGACATGCTTGAGCAACGCAC (SEQ ID NO:30)-3'.

The PCR product was digested with restriction enzymes EcoR1 and Acc65I, and ligated into pEGFP-C1 (Clontech; Palo Alto; GenBank Accession number

U55763) digested with EcoR1 and Acc65I. This produces an EGFP-Smad2 fusion (SEQ ID NOs: 7 and 8) under the control of a CMV promoter.

b) The human Smad2 gene (GenBank Accession number: AF027964) was amplified using PCR according to standard protocols with primers

Smad2-top

B4
cont'd

5'-GTGAATTCGACCATGTCGTCCATCTTGCCATTC (SEQ ID NO:31)-3'

and Smad2-bottom/-stop

5'-GTGGTACCCATGACATGCTTGAGCAACGCAC (SEQ ID NO:32)-3'--

Please replace the paragraph beginning on page 49, line 16 with the following amended paragraph:

--EXAMPLE 5 Probes for detection of VASP redistribution.

Useful for monitoring signalling pathways involving rearrangement of cytoskeletal elements, e.g. to identify compounds which modulate the activity of the pathway in living cells. VASP, a phosphoprotein, is a component of cytoskeletal structures, which redistributes in response to signals that affect focal adhesions.

The human VASP gene (GenBank Accession number: Z46389) was amplified using PCR according to standard protocols with primers

VASP-top

5'-GGGAAGCTTCCATGAGCGAGACGGTCATC (SEQ ID NO:33)-3'

and VASP-bottom/+stop

5'-CCCGGATCCTCAGGGAGAACCCCGCTTC (SEQ ID NO:34)-3'--

Please replace the paragraph beginning on page 50, line 4 with the following amended paragraph:

--EXAMPLE 7 Probes for detection of NFkappaB redistribution.

Useful for monitoring signalling pathways leading to activation of NFkappaB, e.g. to identify compounds which modulate the activity of the pathway in living cells.

NFkappaB, an activator of transcription, is a component of signalling pathways that are responsive to a variety of inducers including cytokines, lymphokines, and some immunosuppressive agents.

a) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers NFkappaB-top
5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:35)-3'
and NFkappaB-bottom/+stop

5'-GTGGATCCTTAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:36)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-C1 (Clontech, Palo Alto; GenBank Accession number U55763) digested with Xho1 and BamH1. This produces an EGFP-NFkappaB fusion (SEQ ID NOs:13 and 14) under the control of a CMV promoter.

b) The human NFkappaB p65 subunit gene (GenBank Accession number: M62399) is amplified using PCR according to standard protocols with primers NFkappaB-top
5'-GTCTCGAGCCATGGACGAACTGTTCCCCCTCATC (SEQ ID NO:37)-3'

and NFkappaB-bottom/-stop

5'-GTGGATCCAAGGAGCTGATCTGACTCAGCAG (SEQ ID NO:38)-3'.

The PCR product is digested with restriction enzymes Xho1 and BamH1, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession number U55762) digested with Xho1 and BamH1. This produces an NFkappaB-EGFP fusion (SEQ ID NOS: 15 and 16) under the control of a CMV promoter.--

Please replace the paragraph beginning on page 54, line 1 with the following amended paragraph:

--**EXAMPLE 11 Probes for detection of PKC β 1 redistribution.**

Useful for monitoring signalling pathways involving Protein Kinase C, e.g. to

identify compounds which modulate the activity of the pathway in living cells.

PKCbeta1, a serine/threonine protein kinase, is closely related to PKCalpha and PKCbeta2 but not identical; it is a component of a signalling pathway which is activated by elevation of intracellular calcium concomitant with an increase in diacylglycerol species.

a) The human PKCbeta1 gene (GenBank Accession number: X06318) was amplified using PCR according to standard protocols with primers

PKC β 1-top

GTCTCGAGGCAAGATGGCTGACCC (SEQ ID NO:39)

and PKC β 1-bottom

GTGGATCCCTACACATTAATGACAACTCTGGG (SEQ ID NO:40).--